PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 03 May 1999 (03.05.99)	
International application No. PCT/JP98/03670	**Applicant's or agent's file reference** 660851
International filing date (day/month/year) 19 August 1998 (19.08.98)	**Priority date** (day/month/year) 22 August 1997 (22.08.97)
Applicant KATO, Seishi et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

12 March 1999 (12.03.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Sean Taylor

Telephone No.: (41-22) 338.83.38

Copy for the designated Office (DO/US)

PCT/JP98/03670

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

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NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

AOYAMA, Tamotsu
Aoyama & Partners
IMP Building
3-7, Shiromi 1-chome
Chuo-ku, Osaka-shi
Osaka 540-0001
JAPON

Date of mailing (day/month/year)
26 April 1999 (26.04.99)

Applicant's or agent's file reference
660851

IMPORTANT NOTIFICATION

International application No.
PCT/JP98/03670

International filing date (day/month/year)
19 August 1998 (19.08.98)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address
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Tokyo 125-0054
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State of Residence: JP
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [X] the address [ ] the nationality [ ] the residence

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Kanagawa 214-0037
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State of Nationality: JP
State of Residence: JP
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[X] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
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1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
Susumo Kubo

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
660851		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/JP98/03670	19/08/1998	22/08/1997
International Patent Classification (IPC) or national classification and IPC		
C12N15/12		
Applicant		
SAGAMI CHEMICAL RESEARCH CENTER et al.		

"Express Mail" mailing label number: EE612046770US
Date of Deposit February 7, 2000
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner For Patents, Washington, D.C. 20231
Signature

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
12/03/1999	12. 11. 99
Name and mailing address of the international preliminary examining authority:	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Julia. P Telephone No. +49 89 2399 8410

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/JP98/03670

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-47 as originally filed

Claims, No.:

1-5 as originally filed

Drawings, sheets:

1/4-4/4 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

see separate sheet

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

☐ copy of the earlier application whose priority has been claimed.

☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has

been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

see separate sheet

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-5
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-5
Industrial applicability (IA)	Yes:	Claims	1-5
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

1. Additional remarks to item I :

A "Sequence Listing" has been filed with the present application (letter of 27.11.98 and received on 30.11.98). This "Sequence Listing" comprises SEQ ID No.: 1 to SEQ ID No.: 6 (pages 1-8).

2. Additional remarks to item II :

The priority documents pertaining to the present application were not available at the time of establishing this international preliminary examination report (IPER). Hence, the current assessment is based on the assumption that all claims enjoy priority rights from the filing date of the priority document (22.08.97). If it later turns out that this is not correct, the documents (a) S. Kato, EMBL Database 01.01.98, AC=014532 and (b) S.Kato, EMBL Database 10.09.97, AC=AB006782 cited in the International Search Report (ISR) could become relevant to assess whether the claimed subject matter of the present application satisfies the criteria set forth in Article 33 (1) PCT.

3. Additional remarks to item V :

The present application discloses the amino acid (SEQ ID No.: 2 and 5-6) and nucleic acid (SEQ ID No.: 4 and 5) sequences of an isoform of the human lactose-binding lectin galectin-9 which have been isolated from a human stomach cancer cDNA library (described in WO97/03190). Said isoform differs from the earlier known human galectin-9 sequence in a few amino acid exchanges and a 32 amino acid insertion (SEQ ID No.: 1 aa; SEQ ID No.: 3 bp). It has 355 residues (MW about 40 kDa) with a 69.3% homology to the mouse galectin-9 isoform and by northern blot it has been shown that there is a different tissue distribution between the claimed human galectin-9 isoform (Figure 4) and the known human galectin-9 protein (Figure 3).

The following documents have been cited in the International Search Report (ISR) as being relevant prior art for the claimed subject matter:

i) Ö. Türeci et al., J. Biol. Chem. 1997, Vol. 272 (10), pages 6416-6422 (**D1**) discloses the identification of the human galectin-9 (MW 36 kDa) in the serum of Hodgkin's disease and shows its amino acid and nucleic acid sequences (figure 1) as well as the comparison with other human and rat galectins (figure 2).

ii) J. Wada and Y.S. Kanwar, J. Biol. Chem. 1997, Vol. 272 (9), pages 6078-6086 (**D2**) discloses the amino acid (figures 1-2) and nucleic acid (figure 3) sequences of a mouse galectin-9 and its unique alternate splicing isoform which is exclusively expressed in small intestine and has a 31 amino acid insertion. The amino acid sequence corresponding to the 31 aa insertion of the intestinal isoform disclosed in D2 is not identical to the insertion sequence present in the claimed human galectin-9 isoform but they have a high homology and share several identical overlapping fragments. Furthermore, D2 refers to the rat galectin-9 and to the presence of a rat galectin-9 isoform present exclusively in the intestine and having a 32 residues insertion. In the discussion, reference is made to the presence of the alternate intestinal galectin-9 isoform (with an insertion sequence) as a unique feature of the galectin-9 in general (page 6085). Reference is also made to the identification and isolation of a corresponding human galectin-9 from human Hodgkin's lymphoma library, wherein said human galectin-9 is said to have a 70% homology with the mouse galectin-9.

iii) J. Wada et al., J. Clin. Invest. 1997, Vol. 99(10), pages 2452-2461 (**D3**) refers to the developmental regulation, expression and the possible (apoptotic) biological activity of (mouse) galectin-9. There is one reference to the presence of a long splicing isoform of galectin-9, exclusively expressed in the small intestine and having a 31 amino acid insertion (actually by reference to D2) (page 2452). However, there is no further explicit mention of said isoform and the results shown in the document do not seem to distinguish between the galectin-9 and its intestinal isoform (probes, peptide used for raising polyclonal antibodies, etc... are not specific for the intestinal isoform but for both galectin-9 and said isoform).

iv) WO-A-97/03190 (**D4**) discloses the identification and isolation of the human galectin-4 which is shown to be expressed specifically in the stomach and intestines. Thus, the human stomach cancer cDNA library used in the document allows the skilled person to detect human galectin genes expressed specifically in the intestines.

None of these documents cited in the ISR discloses the specific amino acid (SEQ ID No.: 2 and 5-6) and nucleic acid (SEQ ID No.: 4 and 5) sequences of the human lactose-binding lectin galectin-9 isoform of the present application. Thus, the subject matter of claims 1-5 is considered to be novel (Article 33 (2) PCT). However, and in view of the

general teachings of D2, in particular the presence of a unique intestinal galectin-9 isoform for all known galectin-9 (namely mouse and rat), the IPEA considers that the skilled person would have obviously try to identify and isolate the corresponding human intestinal galectin-9 isoform. In doing so, the skilled person had more than a reasonable expectation of success (D2 discloses a suitable method, probes, identification, etc...; D4 provides a suitable human cDNA library too). Thus, the subject matter of claims 1-5 is not considered to amount to an inventive contribution over the combined teachings of D2 with D4 and the general knowledge of the skilled person. Claims 1-5 do not fulfil the requirements of Article 33 (3) PCT.

4. Additional remarks to item VIII :

The following objection is also raised under **Article 6 PCT** concerning the clarity of the claims and the consistency with the description (see PCT Gazette, Special Issue, PCT International Preliminary Examination Guidelines, as in force from 09.10.98, Section IV, III-4.3). The description refers to different products such as peptides fragments of the disclosed intestinal galectin-9 isoform, mutants and variants thereof, species homologues and allelic variants thereof, cDNA fragments, polynucleotides, etc... as embodiments of the present invention, which certainly fall outside the subject matter covered by the claims and which in view of the prior art concerned with the normal known human galectin-9 are neither novel (Article 33(2) PCT) nor inventive (Article 33 (3) PCT).

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
660851		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/JP 98/ 03670	19/08/1998	22/08/1997

Applicant

SAGAMI CHEMICAL RESEARCH CENTER et al.

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

"Express Mail" mailing label number: EL332462744US
Date of Deposit February 17, 2000
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner For Patents, Washington, D.C. 20231
Signature

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [X] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [X] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title**,
   - [ ] the text is approved as submitted by the applicant
   - [X] the text has been established by this Authority to read as follows:

   HUMAN GALECTIN-9-LIKE PROTEINS AND CDNAS ENCODING THESE PROTEINS

5. With regard to the **abstract**,
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. –
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/JP 98/03670

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TURECI O ET AL: "Molecular definition of a novel human galectin which is immunogenic in patients with Hodgkin's disease." JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 MAR 7) 272 (10) 6416-22. JOURNAL CODE: HIV. ISSN: 0021-9258., XP002065487 United States cited in the application see the whole document --- -/--	1-5

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 January 1999	22/01/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kania, T

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WADA J ET AL: "Identification and characterization of galectin - 9, a novel beta-galactoside-binding mammalian lectin." JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 FEB 28) 272 (9) 6078-86. JOURNAL CODE: HIV. ISSN: 0021-9258., XP002089578 United States cited in the application see page 6082, column 2, line 11FF.; figure 1B	1-5
A	WADA J ET AL: "Developmental regulation, expression, and apoptotic potential of galectin - 9, a beta-galactoside binding lectin." JOURNAL OF CLINICAL INVESTIGATION, (1997 MAY 15) 99 (10) 2452-61. JOURNAL CODE: HS7. ISSN: 0021-9738., XP002089579 United States see the whole document	1-5
A	WO 97 03190 A (KATO SEISHI ;SEKINE SHINGO (JP); KAMATA KOUJU (JP); SAGAMI CHEM RE) 30 January 1997 cited in the application see the whole document	1-5
P,X	KATO S.: "AC 014532" EMBL DATABASE,1 January 1998, XP002089580 Heidelberg see the whole document	1,2
P,X	KATO S.: "AC AB006782" EMBL DATABASE,10 September 1997, XP002089581 Heidelberg see the whole document	3-5

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/JP 98/03670

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9703190 A	30-01-1997	EP 0841393 A	13-05-1998

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/12, C07K 14/47 — A1

(11) International Publication Number: **WO 99/10490**

(43) International Publication Date: 4 March 1999 (04.03.99)

(21) International Application Number: PCT/JP98/03670

(22) International Filing Date: 19 August 1998 (19.08.98)

(30) Priority Data:
9/226468 22 August 1997 (22.08.97) JP

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(81) Designated States: AU, CA, JP, MX, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: HUMAN GALECTIN–9–LIKE PROTEINS AND CDNAS ENCODING THESE PROTEINS

(57) Abstract

Cloning of human cDNAs coding for galectin–9–like proteins, expression with *Escherichia coli* of proteins encoded by these human cDNAs, and determination of the lactose–binding activity of these expression products.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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DESCRIPTION

HUMAN GALECTIN-9-LIKE PROTEINS AND CDNAS ENCODING THESE PROTEINS

FIELD OF THE INVENTION

The present invention relates to galactin-9-like proteins and cDNAs coding for these proteins. The proteins of the present invention can be employed as pharmaceuticals or reagents for sugar chain researches. The human cDNAs of the present invention can be utilized as probes for the gene diagnosis and gene sources for the gene therapy. Furthermore, the cDNAs can be utilized as gene sources for large-scale production of the proteins encoded by said cDNAs.

BACKGROUND OF THE INVENTION

Galectins are the general term for animal lectins binding to galactose. Animal lectins exist in many sites such as the cytoplasm, the nucleus, the cell membrane surface, etc., and are considered to be associated with the cell proliferation, the differentiation, the canceration, the metastasis, the immunity, and so on [Dickamer, K., Annu. Rev. Cell Biol. 9: 237-264 (1993)]. There have been heretofore known 9 kinds of galectins, namely galectin-1 to galectin-9.

Galectin-9 is a lectin that has been identified as an antigenic protein reacting with an antibody contained in the serum of patients with Hodgkin's disease [Tureci, O., J. Biol. Chem.

272: 6416-6422 (1997)]. Galectin-9 has a structure where two sugar chain-binding domains are connected by a linker peptide, in the same manner as in galectin-4 and galectin-8. The true role of galectin-9 in the living body has not yet been completely clarified, but it has been considered to be involved in the adhesion between cells. Although two types of galectin-9 having different molecular weights in mice have been reported [Wada, J. and Kanwar, Y. S., J. Biol. Chem. 272: 6078-6086 (1997)], there have not been reported such isoforms in the human.

SUMMARY OF THE INVENTION

The object of the present invention is to provide human galactin-9-like proteins and cDNAs encoding these proteins.

As the result of intensive studies, the present inventors have been successful in cloning of human cDNAs coding for galectin-9-like proteins, thereby completing the present invention. In other words, the present invention provides galectin-9-like proteins, namely proteins containing the amino acid sequences represented by Sequence No. 1 and Sequence No. 2. Moreover, the present invention provides cDNAs coding for the above-mentioned proteins and containing the base sequences represented by Sequence No. 3 to Sequence No. 5.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1: A figure depicting the structure of plasmid pHP01461.

Figure 2: A figure illustrating the results of analysis by SDS-PAGE of (1) a human galectin-9-like protein that is translated

in vitro and (2) a human galectin-9-like protein that is bound to the lactose column.

Figure 3: A figure illustrating the result of northern blot hybridization that was carried out by using the cDNA fragment as a probe.

Figure 4: A figure illustrating the result of northern blot hybridization that was carried out by using the oligonucleotide in the inserted portion as a probe.

BEST MODE FOR CARRYING OUT THE INVENTION

The proteins of the present invention can be obtained, for example, by a method for isolation from human organs, cell lines, etc., a method for preparation of peptides by the chemical synthesis, or a method for production with the recombinant DNA technology using the DNAs coding for the human galactin-9-like proteins of the present invention, wherein the method for obtainment by the recombinant DNA technology is employed preferably. For instance, in vitro expression can be achieved by preparation of an RNA by in vitro transcription from a vector having one of cDNAs of the present invention, followed by in vitro translation using this RNA as a template. Also, recombination of the translation region into a suitable expression vector by the method known in the art leads to expression of a large amount of the encoded protein by using *Escherichia coli*, *Bacillus subtilis*, yeasts, animal cells, and so on.

In the case in which a protein of the present invention is expressed by a microorganism such as *Escherichia coli*, a

recombinant expression vector bearing the translation region in the cDNA of the present invention is constructed in an expression vector having an origin, a promoter, a ribosome-binding site, a cDNA-cloning site, a terminator etc., which can be replicated in the microorganism, and, after transformation of the host cells with said expression vector, the thus-obtained transformant is incubated, whereby the protein encoded by said cDNA can be produced on a large scale in the microorganism. Alternatively, a fusion protein with another protein can be expressed. Only a protein portion coding for said cDNA can be obtained by cleavage of said fusion protein with an appropriate protease. Said fusion protein, provided that it possesses the lactose-binding activity, shall come within the scope of the present invention.

In the case in which a protein of the present invention is subjected to secretory expression in animal cells, the protein of the present invention can be produced by extracellular secretion, when the translation region of said cDNA is subjected to recombination to an expression vector for animal cells that has a promoter, a splicing region, a poly(A) addition site, etc., followed by introduction into the animal cells.

The proteins of the present invention include peptide fragments (more than 5 amino acid residues) containing any partial amino acid sequence in the amino acid sequence represented by Sequence No. 1. These peptide fragments can be utilized as antigens for preparation of antibodies. Hereupon, the proteins of the present invention are secreted in an extracellular manner. Since a portion capable of binding sugar chains exists in the amino acid

sequence, proteins where sugar chains are added can be obtained by expression in appropriate animal cells. Accordingly, such proteins or peptides wherein sugar chains are added shall come within the scope of the present invention.

The DNAs of the present invention include all DNAs coding for the above-mentioned proteins. Said DNAs can be obtained by using a method by chemical synthesis, a method by cDNA cloning, and so on.

The human cDNAs of the present invention can be cloned from cDNA libraries of the human cell origin. These cDNA libraries are constructed by using as templates poly(A)$^+$ RNAs extracted from human cells. The human cells may be cells delivered from the human body, for example, by the operation or may be the culture cells. A poly(A)$^+$ RNA isolated from a stomach cancer tissue is used in Examples. The cDNAs can be synthesized by using any method selected from the Okayama-Berg method [Okayama, H. and Berg, P., Mol. Cell. Biol. 2: 161-170 (1982)], the Gubler-Hoffman method [Gubler, U. and Hoffman, J. Gene 25: 263-269 (1983)], and so on, but it is preferred to use the capping method [Kato, S. et al., Gene 150: 243-250 (1994)] in order to obtain a full-length clone in an effective manner. The identification of the cDNAs is carried out by the determination of the whole base sequence by the sequencing, the search of known proteins having sequences analogous to the amino acid sequence predicted from the base sequence, expression of proteins by in vitro translation, expression by *Escherichia coli*, and the activity measurement of expressed products. The activity measurement is carried out by identification of the

binding with lactose.

The cDNAs of the present invention are characterized by containing the base sequence represented by Sequence No. 3 or Sequence No. 4. For example, that represented by Sequence No. 5 possesses a 1725-bp base sequence with a 1068-bp open reading frame. This open reading frame codes for a protein consisting of 355 amino acid residues. This protein possesses such a high 69.3% analogy to the mouse galectin-9-like isoform in the amino acid sequence level.

Hereupon, the same clones as the cDNAs of the present invention can be easily obtained by screening of the human cDNA libraries constructed from the human cells by the use of an oligonucleotide probe synthesized on the basis of the cDNA base sequence described in Sequence No. 3.

In general, the polymorphism due to the individual difference is frequently observed in human genes. Accordingly, any cDNA that is subjected to insertion or deletion of one or plural nucleotides and/or substitution with other nucleotides in Sequence No. 3 to Sequence No. 5 shall come within the scope of the present invention.

In a similar manner, any protein that is formed by these modifications comprising insertion or deletion of one or plural amino acids and/or substitution with other amino acids shall come within the scope of the present invention, as far as the protein possesses the human galectin-9-like activity.

The cDNAs of the present invention include cDNA fragments (more than 10 bp) containing any partial base sequence in the base

sequence represented by Sequence No. 3. Also, DNA fragments consisting of a sense chain and an anti-sense chain shall come within this scope. These DNA fragments can be utilized as the probes for the gene diagnosis.

The present invention also provides genes corresponding to the polynucleotide sequences disclosed herein. "Corresponding genes" are the regions of the genome that are transcribed to produce the mRNAs from which cDNA polynucleotide sequences are derived and may include contiguous regions of the genome necessary for the regulated expression of such genes. Corresponding genes may therefore include but are not limited to coding sequences, 5' and 3' untranslated regions, alternatively spliced exons, introns, promoters, enhancers, and silencer or suppressor elements. The corresponding genes can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include the preparation of probes or primers from the disclosed sequence information for identification and/or amplification of genes in appropriate genomic libraries or other sources of genomic materials. An "isolated gene" is a gene that has been separated from the adjacent coding sequences, if any, present in the genome of the organism from which the gene was isolated.

Organisms that have enhanced, reduced, or modified expression of the gene(s) corresponding to the polynucleotide sequences disclosed herein are provided. The desired change in gene expression can be achieved through the use of antisense polynucleotides or ribozymes that bind and/or cleave the mRNA

transcribed from the gene (Albert and Morris, 1994, Trends Pharmacol. Sci. 15(7): 250-254; Lavarosky et al., 1997, Biochem. Mol. Med. 62(1): 11-22; and Hampel, 1998, Prog. Nucleic Acid Res. Mol. Biol. 58: 1-39; all of which are incorporated by reference herein). Transgenic animals that have multiple copies of the gene(s) corresponding to the polynucleotide sequences disclosed herein, preferably produced by transformation of cells with genetic constructs that are stably maintained within the transformed cells and their progeny, are provided. Transgenic animals that have modified genetic control regions that increase or reduce gene expression levels, or that change temporal or spatial patterns of gene expression, are also provided (see European Patent No. 0 649 464 B1, incorporated by reference herein). In addition, organisms are provided in which the gene(s) corresponding to the polynucleotide sequences disclosed herein have been partially or completely inactivated, through insertion of extraneous sequences into the corresponding gene(s) or through deletion of all or part of the corresponding gene(s). Partial or complete gene inactivation can be accomplished through insertion, preferably followed by imprecise excision, of transposable elements (Plasterk, 1992, Bioessays 14(9): 629-633; Zwaal et al., 1993, Proc. Natl. Acad. Sci. USA 90(16): 7431-7435; Clark et al., 1994, Proc. Natl. Acad. Sci. USA 91(2): 719-722; all of which are incorporated by reference herein), or through homologous recombination, preferably detected by positive/negative genetic selection strategies (Mansour et al., 1988, Nature 336: 348-352; U.S. Patent Nos. 5,464,764; 5,487,992;

5,627,059; 5,631,153; 5,614, 396; 5,616,491; and 5,679,523; all of which are incorporated by reference herein). These organisms with altered gene expression are preferably eukaryotes and more preferably are mammals. Such organisms are useful for the development of non-human models for the study of disorders involving the corresponding gene(s), and for the development of assay systems for the identification of molecules that interact with the protein product(s) of the corresponding gene(s).

Where the protein of the present invention is membrane-bound (e.g., is a receptor), the present invention also provides for soluble forms of such protein. In such forms part or all of the intracellular and transmembrane domains of the protein are deleted such that the protein is fully secreted from the cell in which it is expressed. The intracellular and transmembrane domains of proteins of the invention can be identified in accordance with known techniques for determination of such domains from sequence information.

Proteins and protein fragments of the present invention include proteins with amino acid sequence lengths that are at least 25%(more preferably at least 50%, and most preferably at least 75%) of the length of a disclosed protein and have at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% or 95% identity) with that disclosed protein, where sequence identity is determined by comparing the amino acid sequences of the proteins when aligned so as to maximize overlap and identity while minimizing sequence gaps. Also included in the present invention are proteins and protein

fragments that contain a segment preferably comprising 8 or more (more preferably 20 or more, most preferably 30 or more) contiguous amino acids that shares at least 75% sequence identity (more preferably, at least 85% identity; most preferably at least 95% identity) with any such segment of any of the disclosed proteins.

Species homologs of the disclosed polynucleotides and proteins are also provided by the present invention. As used herein, a "species homologue" is a protein or polynucleotide with a different species of origin from that of a given protein or polynucleotide, but with significant sequence similarity to the given protein or polynucleotide, as determined by those of skill in the art. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from the desired species.

The invention also encompasses allelic variants of the disclosed polynucleotides or proteins; that is, naturally-occurring alternative forms of the isolated polynucleotide which also encode proteins which are identical, homologous, or related to that encoded by the polynucleotides.

The invention also includes polynucleotides with sequences complementary to those of the polynucleotides disclosed herein.

The present invention also includes polynucleotides capable of hybridizing under reduced stringency conditions, more preferably stringent conditions, and most preferably highly stringent conditions, to polynucleotides described herein. Examples of stringency conditions are shown in the table below:

highly stringent conditions are those that are at least as stringent as, for example, conditions A-F; stringent conditions are at least as stringent as, for example, conditions G-L; and reduced stringency conditions are at least as stringent as, for example, conditions M-R.

Table 1

Stringency Condition	Polynucleotide Hybrid	Hybrid Length (bp)‡	Hybridization Temperature and Buffer†	Wash Temperature and Buffer†
A	DNA : DNA	≥50	65℃; 1×SSC -or- 42℃; 1×SSC,50% formamide	65℃; 0.3×SSC
B	DNA : DNA	<50	T_B*; 1×SSC	T_B*; 1×SSC
C	DNA : RNA	≥50	67℃; 1×SSC -or- 45℃; 1×SSC,50% formamide	67℃; 0.3×SSC
D	DNA : RNA	<50	T_D*; 1×SSC	T_D*; 1×SSC
E	RNA : RNA	≥50	70℃; 1×SSC -or- 50℃; 1×SSC,50% formamide	70℃; 0.3×SSC
F	RNA : RNA	<50	T_F*; 1×SSC	T_F*; 1×SSC
G	DNA : DNA	≥50	65℃; 4×SSC -or- 42℃; 4×SSC,50% formamide	65℃; 1×SSC
H	DNA : DNA	<50	T_H*; 4×SSC	T_H*; 4×SSC
I	DNA : RNA	≥50	67℃; 4×SSC -or- 45℃; 4×SSC,50% formamide	67℃; 1×SSC
J	DNA : RNA	<50	T_J*; 4×SSC	T_J*; 4×SSC
K	RNA : RNA	≥50	70℃; 4×SSC -or- 50℃; 4×SSC,50% formamide	67℃; 1×SSC
L	RNA : RNA	<50	T_L*; 2×SSC	T_L*; 2×SSC
M	DNA : DNA	≥50	50℃; 4×SSC -or- 40℃; 6×SSC,50% formamide	50℃; 2×SSC
N	DNA : DNA	<50	T_N*; 6×SSC	T_N*; 6×SSC
O	DNA : RNA	≥50	55℃; 4×SSC -or- 42℃; 6×SSC,50% formamide	55℃; 2×SSC
P	DNA : RNA	<50	T_P*; 6×SSC	T_P*; 6×SSC
Q	RNA : RNA	≥50	60℃; 4×SSC -or- 45℃; 6×SSC,50% formamide	60℃; 2×SSC
R	RNA : RNA	<50	T_R*; 4×SSC	T_R*; 4×SSC

‡ : The hybrid length is that anticipated for the hybridized region(s) of the hybridizing polynucleotides. When hybridizing a polynucleotide to a target polynucleotide of unknown sequence, the hybrid length is assumed to be that of the hybridizing polynucleotide. When polynucleotides of known sequence are hybridized, the hybrid length can be determined by

aligning the sequences of the polynucleotides and identifying the region or regions of optimal sequence complementarity.

†: SSPE (1×SSPE is 0.15M NaCl, 10mM NaH_2PO_4, and 1.25mM EDTA, pH7.4) can be substituted for SSC (1×SSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes after hybridization is complete.

*T_B - T_R: The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10℃ less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, T_m(℃)=2(#of A + T bases) + 4(# of G + C bases). For hybrids between 18 and 49 base pairs in length, T_m(℃)=81.5 + 16.6($\log_{10}$[Na^+]) + 0.41 (%G+C) - (600/N), where N is the number of bases in the hybrid, and [Na^+] is the concentration of sodium ions in the hybridization buffer ([Na^+] for 1×SSC=0.165M).

Additional examples of stringency conditions for polynucleotide hybridization are provided in Sambrook, J., E.F. Fritsch, and T. Maniatis, 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, chapters 9 and 11, and Current Protocols in Molecular Biology, 1995, F.M. Ausubel et al., eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4, incorporated herein by reference.

Preferably, each such hybridizing polynucleotide has a length that is at least 25% (more preferably at least 50%, and most preferably at least 75%) of the length of the polynucleotide of the present invention to which it hybridizes, and has at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% or 95% identity) with the polynucleotide of the present invention to which it hybridizes, where sequence identity is determined by comparing the sequences of the hybridizing polynucleotides when aligned so as to maximize overlap and identity while minimizing sequence gaps.

EXAMPLES

The present invention is embodied in more detail by the following examples, but this embodiment is not intended to restrict the present invention. The basic operations and the enzyme reactions with regard to the DNA recombination are carried out according to the literature ["Molecular Cloning. A Laboratory Manual", Cold Spring Harbor Laboratory, 1989]. Unless otherwise stated, restrictive enzymes and a variety of modification enzymes to be used were those available from TAKARA SHUZO. The manufacturer's instructions were used for the buffer compositions as well as for the reaction conditions, in each of the enzyme reactions.

(1) cDNA Cloning

Clone HP01461 was obtained as the result of a large-scale sequencing of cDNA clones selected from the cDNA library of human stomach cancer cells (described in WO97/03190). The present clone has a structure consisting of an 81-bp 5'-nontranslation region, a 1068-bp open reading frame, a 576-bp 3'-nontranslation region, and an 83-bp poly(A) tail (Sequence No. 5). The open reading frame codes for a protein consisting of 355 amino acid residues and the search of the protein data base using this sequence has revealed the presence of a high analogy to the amino acid sequences of human galectin-9 and mouse galectin-9 isoform. Table 2 shows the comparison of the amino acid sequence between the human galectin-like protein (HS) of the present invention and the human galectin-9 (G9), while Table 3 shows the comparison of the amino acid sequence between the human galectin-like protein (HS) of the present invention and the mouse galectin-9 isoform (MM). Therein,

the marks of -, *, and . represent a gap, an amino acid residue identical with the protein of the present invention, and an amino acid residue analogous to the protein of the present invention, respectively. Comparison of the protein of the present invention with human galectin-9 has revealed that there are differences at the following 6 sites. That is to say, they are lysine at position 88 (arginine in G), insertion of glycine at position 96, serine at position 135 (phenylalanine in G9), insertion of 32 amino acid residues from position 149 to position 180, proline at position 270 (leucine in G9), and glutamic acid at position 313 (glycine in G9). Because comparison of the protein of the present invention with the mouse galectin-9 isoform has revealed that the protein of the present invention has a sequence that is longer only by 2 amino acid residues and a 69.3% analogy is shown in the entire region, the protein of the present invention is considered to be a homologue of the mouse galectin-9 isoform.

Table 2

```
HS MAFSGSQAPYLSPAVPFSGTIQGGLQDGLQITVNGTVLSSSGTRFAVNFQTGFSGNDIAF
   ************************************************************
G9 MAFSGSQAPYLSPAVPFSGTIQGGLQDGLQITVNGTVLSSSGTRFAVNFQTGFSGNDIAF
HS HFNPRFEDGGYVVCNTRQNGSWGPEERKTHMPFQKGMPFDLCFLVQSSDFKVMVNGILFV
   ***************************. ******* ************************
G9 HFNPRFEDGGYVVCNTRQNGSWGPEERRTHMPFQK-MPFDLCFLVQSSDFKVMVNGILFV
HS QYFHRVPFHRVDTISVNGSVQLSYISFQNPRTVPVQPAFSTVPFSQPVCFPPRPRGRRQK
   ************** **************
G9 QYFHRVPFHRVDTIFVNGSVQLSYISFQ------------------------------
HS PPGVWPANPAPITQTVIHTVQSAPGQMFSTPAIPPMMYPHPAYPMPFITTILGGLYPSKS
   ************************************************************
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```
G9 PPGVWPANPAPITQTVIHTVQSAPGQMFSTPAIPPMMYPHPAYPMPFITTILGGLYPSKS
HS ILLSGTVLPSAQRFHINLCSGNHIAFHLNPRFDENAVVRNTQIDNSWGSEERSLPRKMPF
   ****************************** ******************************
G9 ILLSGTVLPSAQRFHINLCSGNHIAFHLNLRFDENAVVRNTQIDNSWGSEERSLPRKMPF
HS VRGQSFSVWILCEAHCLKVAVDGQHLFEYYHRLRNLPTINRLEVGGDIQLTHVQT
   ***********.****************************************
G9 VRGQSFSVWILCGAHCLKVAVDGQHLFEYYHRLRNLPTINRLEVGGDIQLTHVQT
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Table 3

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HS MAFSGSQAPYLSPAVPFSGTIQGGLQDGLQITVNGTVLSSSGTRFAVNFQTGFSGNDIAF
   **. ..*.**..* .**.*.******.***.*..**. .* . **.****..*.******
MM MALFSAQSPYINPIIPFTGPIQGGLQEGLQVTLQGTT-KSFAQRFVVNFQNSFNGNDIAF
HS HFNPRFEDGGYVVCNTRQNGSWGPEERKTHMPFQKGMPFDLCFLVQSSDFKVMVNGILFV
   *******.********.*** ******* .*********.******.*.******  .**
MM HFNPRFEEGGYVVCNTKQNGQWGPEERKMQMPFQKGMPFELCFLVQRSEFKVMVNKKFFV
HS QYFHRVPFHRVDTISVNGSVQLSYISFQNPRTVPVQPAFSTVPFSQPVCFPPRPRGRRQK
   ** ****.* ****.*.*...**.*.***. ..***..***..***** **. *.**.**
MM QYQHRVPYHLVDTIAVSGCLKLSFITFQNS-AAPVQHVFSTLQFSQPVQFPRTPKGRKQK
HS PPGVWPANPAPITQTVIHTVQSAPGQMFSTPAIPPMMYPHPAYPMPFITTILGGLYPSKS
   ... .**..**..**.** *.*.********.***..** ***..** *.* .*******
MM TQNFRPAHQAPMAQTTIHMVHSTPGQMFSTPGIPPVVYPTPAYTIPFYTPIPNGLYPSKS
HS ILLSGTVLPSAQRFHINLCSGNHIAFHLNPRFDENAVVRNTQIDNSWGSEERSLPRKMPF
   *..**.***.* ****** .*..*********.**********.**** *****  .***
MM IMISGNVLPDATRFHINLRCGGDIAFHLNPRFNENAVVRNTQINNSWGQEERSLLGRMPF
HS VRGQSFSVWILCEAHCLKVAVDGQHLFEYYHRLRNLPTINRLEVGGDIQLTHVQT
    *********.**.**.****.***. ******.**..** ***.**********
MM SRGQSFSVWIICEGHCFKVAVNGQHMCEYYHRLKNLQDINTLEVAGDIQLTHVQT
```

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(2) Protein Synthesis by In Vitro Translation

Vector pHP01461 bearing the cDNA of the present invention was used for in vitro translation with a T_NT rabbit reticulocyte lysate kit (Promega). In this case, [^{35}S]methionine was added to label the expression product with a radioisotope. Each of the

reactions was carried out according to the protocols attached to the kit. Two micrograms of plasmid pHP01416 was reacted at 30℃ for 90 minutes in a total 100 μl volume of the reaction solution containing 50 μl of T_NT rabbit reticulocyte lysate, 4 μl of a buffer solution (attached to kit), 2 μl of an amino acid mixture (methionine-free), 8 μl of [^{35}S]methionine (Amersham) (0.37 MBq/μl), 2 μl of T7RNA polymerase, and 80 U of RNasin. To 3 μl of the resulting reaction solution was added 2 μl of the SDS sampling buffer (125 mM Tris-hydrochloric acid buffer, pH 6.8, 120 mM 2-mercaptoethanol, 2% SDS solution, 0.025% bromophenol blue, and 20% glycerol) and the resulting mixture was heated at 95℃ for 3 minutes and then subjected to SDS-polyacrylamide gel electrophoresis. Determination of the molecular weight of the translation product by carrying out the autoradiograph indicated that the cDNA of the present invention yielded the translation product with the molecular mass of about 40 kDa (Figure 2). This value is consistent with the molecular weight of 39,517 predicted for the putative protein from the base sequence represented by Sequence No. 2, thereby indicating that this cDNA certainly codes for the protein represented by Sequence No. 2.

(3) Measurement of Lactose-Binding Activity of In-Vitro Translation Product

After 100 ml of a Sepharose-4B gel suspension (Pharmacia) was washed well with 0.5 M sodium carbonate, the gel was suspended in 100 ml of 0.5 M sodium carbonate. Thereto was added 10 ml of vinyl sulfone and the resulting mixture was gently stirred at room temperature for one hour. After washing with 0.5 M sodium carbonate,

the gel was suspended in a solution of 10% lactose and 0.5 M sodium carbonate, and the resulting suspension was stirred gently overnight at room temperature. The resulting gel was washed in order with 0.5 M sodium carbonate, water, and 0.05 M phosphate buffer (pH 7.0). The thus-obtained lactosyl-Sepharose-4B gel was stored at 4℃ in the 0.05 M phosphate buffer (pH 7.0) containing 0.02% sodium azide.

By chromatography of 100 µl of the in-vitro translation solution on the previously-prepared lactosyl-Sephaarose-4B column (a bed volume of 4.5 ml), the column was washed with 20 ml of a column buffer solution for lactose column (20 mM Tris-hydrochloric acid buffer, pH 7.5, 2 mM EDTA, 150 mM NaCl, 4 mM 2-mercaptoethanol, and 0.01% Triton X-100) and then eluted with 20 ml of the column buffer solution containing 0.3 M lactose. As the result, it is indicated that the protein of the present invention possesses the lactose-binding activity from the observation that the 40-kDa translation product was contained in the eluates (Figure 2).

(4) Expression of Galectin-9-like Protein by *Escherichia coli* and Lactose-Binding Activity

After digestion of 1 µg of plasmid pHP01461 with 20 units of EcoRI and 20 units of NotI, followed by electrophoresis on 0.8% agarose gel, an about 1.7-kbp DNA fragment was cut off from the gel. Then, after digestion of 1 µg of pET21a (Novagen),an expression vector for *Escherichia coli*, with 20 units of EcoRI and 20 units of NotI, followed by electrophoresis on 0.8% agarose gel, an about 5.3-kbp DNA fragment was cut off from the gel. Both

DNA fragments were ligated by using a ligation kit and then *Escherichia coli* JM109 was transformed. Plasmid pET-1461 was prepared from the transformant and the objective recombinant was identified by the restriction enzyme cleavage map.

Two strands of an oligonucleotide primer PR1 (5'-CGCATATGGCCTTCAGCGGTTCCCAGGC-3') and PR2 (5'-AACGGCACCGTGGAGAAGGCAGGCTGAACA-3') were synthesized by using a DNA synthesizer (Applied Biosystems) according to the attached protocol. The 5'-tanslation region in the cDNA was amplified with the PCR kit (TAKARA SHUZO) using 1 ng of plasmid pHP01461 as well as 100 pmole each of primers PR1 and PR2. After phenol extraction and ethanol extraction, followed by digestion with 20 units of SacI and NdeI, the reaction product was subjected to 1.2% agarose electrophoresis to cut off an about 320-bp DNA fragment for purification.

After digestion of 1 μg of plasmid pET-1461 with 20 units of SacI and NdeI, followed by electrophoresis on 0.8% agarose gel, a 3.8-kbp DNA fragment was cut off from the gel. This DNA fragment and the about 320-bp DNA fragment prepared previously by PCR were ligated by using a ligation kit and then *Escherichia coli* BL21 (DE3) was transformed. Plasmid pET-1461 was prepared from the transformant and the objective recombinant was identified by the restriction enzyme cleavage map.

A suspension of 2 ml of an overnight-incubated liquid of pET1461/BL21 (DE3) in 100 ml of the LB culture medium containing 100 μg/ml of ampicillin was incubated in a shaker at 37℃ and isopropylthiogalactoside was added so as to make 1 mM when A_{600}

reached about 0.5. After further incubation at 37℃ for 3 hours, the mycelia collected by centrifugation were suspended in 25 ml of the column buffer for lactose column. After sonication, the suspension was centrifuged and the supernatant was charged into the previously-prepared, lactosyl-Sepharose-4B column (a 2-ml bed volume). The column was washed with 10 ml of the column buffer for lactose column and then eluted with 5 ml of the column buffer containing 0.3 M lactose. The SDS-polyacrylamide electrophoresis of the eluted protein indicated the presence of a single band at the position of 40 kDa. This molecular mass value is consistent with the molecular weight predicted for the human galectin-9-like protein. That is to say, the human galectin-9-like protein expressed by *Escherichia coli* was indicated to possess the lactose-binding activity.

(5) Northern Blot Hybridization

Northern blot hybridization was carried out in order to examine the expression pattern in the human tissues. Filters where poly(A)$^+$ RNAs isolated from each of human tissues are blotted were purchased from Clontech. After digestion of plasmid pHP01049 with ApaLI and BstXI, followed by agarose-gel electrophoresis to isolate a cDNA fragment, labeling with [^{32}P]dCTP (Amersham) was carried out by using a random primer labeling kit (TAKARA SHUZO). Furthermore, the inserted portion was subjected to the terminal ^{32}P-labeling with a synthetic oligonucleotide 5′-AACGGCACCGTGGAGAAGGCAGGCTGAGCA-3′ using T4 polynucleotide kinase. The hybridization was carried out by using a solution attached to the blot paper according to the protocol.

In the case in which the cDNA fragment was employed as a probe, the strongest expression was observed in the peripheral blood and, besides, expression was observed in the heart, the placenta, the lung, the spleen, the thymus, the ovary, the small intestine, and the large intestine. In each case, the size of the transcription product was about 2 kb (Figure 3). On the other hand, in the case in which the inserted portion was used as a probe, a different result was obtained (Figure 4). The about 2kb band was the most intense in the small intestine and the large intestine with weak expression being observed in the lung and the peripheral blood. Beside the band of this size, a strong band of less than 1 kb was observed in the liver and also a band of about 2.4 kb was observed in the kidney. In this way, the expression pattern of human galectin-9 is different in the case in which the inserted portion was used as a probe, so that the proteins of the present invention are indicated to undergo an expression control different from that of known galectin-9 and, also, are predicted to be different in their function.

The present invention provides human cDNAs coding for galectin-9-like proteins and proteins encoded by these human cDNAs. Said recombinant proteins can be expressed in large amounts by utilizing the cDNAs of the present invention. Said recombinant proteins can be employed as pharmaceuticals/research reagents.

In addition to the activities and uses described above, the polynucleotides and proteins of the present invention may exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified below. Uses

or activities described for proteins of the present invention may be provided by administration or use of such proteins or by administration or use of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA).

Research Uses and Utilities

The polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on Southern gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; for selecting and making oligomers for attachment to a "gene chip" or other support, including for examination of expression patterns; to raise anti-protein antibodiesusing DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such

as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

The proteins provided by the present invention can similarly be used in assay to determine biological activity, including in a panel of multiple proteins for high-throughput screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Where the protein binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the protein can be used to identify the other protein with which binding occurs or to identify inhibitors of the binding interaction. Proteins involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

Any or all of these research utilities are capable of being developed into reagent grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known

to those skilled in the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E.F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S.L. and A.R. Kimmel eds., 1987.

Nutritional Uses

Polynucleotides and proteins of the present invention can also be used as nutritional sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In such cases the protein or polynucleotide of the invention can be added to the feed of a particular organism or can be administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the protein or polynucleotide of the invention can be added to the medium in or on which the microorganism is cultured.

Cytokine and Cell Proliferation/Differentiation Activity

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of

a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Bertagnolli et al., J. Immunol. 145:1706-1712, 1990; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Bertagnolli, et al., J. Immunol. 149:3778-3783, 1992; Bowman et al., J. Immunol. 152: 1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A.M. and Shevach, E.M. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human Interferon γ, Schreiber, R.D. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L.S. and Lipsky, P.E. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988; Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse and human interleukin 6-Nordan, R. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11 - Bennett, F., Giannotti, J., Clark, S.C. and Turner, K. J. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9 - Ciarletta, A., Giannotti, J., Clark,S.C. and Turner, K.J. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte

Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

Immune Stimulating or Suppressing Activity

A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial orfungal infections, or may result from autoimmune disorders. More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, Leishmania spp., malaria spp. and various fungal infections such as candidiasis. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally may be desirable, i.e., in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue

disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitis, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using a protein of the present invention.

Using the proteins of the invention it may also be possible to immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions

(including without limitation B lymphocyte antigen functions (such as , for example, B7)), e.g., preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (e.g., B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this matter prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte

antigens.

The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins in vivo as described in Lenschow et al., Science 257:789-792 (1992) and Turka et al., Proc. Natl. Acad. Sci USA, 89:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function in vivo on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block costimulation of T cells by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive

T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythmatosis in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of viral infection. In addition, systemic viral diseases such as influenza, the commoncold, and encephalitis might be alleviated by the administration of stimulatory forms of B lymphocyte antigens systemically.

Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells in vitro with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the in vitro activated T cells into

the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells in vivo.

In another application, up regulation or enhancement of antigen function (preferably B lymphocyte antigen function) may be useful in the induction of tumor immunity. Tumor cells (e.g., sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma) transfected with a nucleic acid encoding at least one peptide of the present invention can be administered to a subject to overcome tumor-specific tolerance in the subject. If desired, the tumor cell can be transfected to express a combination of peptides. For example, tumor cells obtained from a patient can be transfected ex vivo with an expression vector directing the expression of a peptide having B7-2-like activity alone, or in conjunction with a peptide having B7-1-like activity and/or B7-3-like activity. The transfected tumor cells are returned to the patient to result in expression of the peptides on the surface of the transfected cell. Alternatively, gene therapy techniques can be used to target a tumor cell for transfection in vivo.

The presence of the peptide of the present invention having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessary costimulation signal to T cells

to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient amounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (e.g., a cytoplasmic-domain truncated portion) of an MHC class I α chain protein and β_2 microglobulin protein or an MHC class IIα chain protein and an MHC class IIβ chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (e.g., B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing

Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Bowmanet al., J. Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: In vitro antibody production, Mond, J.J. and Brunswick, M. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro

assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine 169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell

commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

Hematopoiesis Regulating Activity

A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned

hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either in-vivo or ex-vivo (i.e., in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al., Molecular and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M.G. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, NY. 1994; Hirayama et al., Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I.K.

and Briddell, R.A. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, NY. 1994; Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R.E. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 1-21, Wiley-Liss, Inc., New York, NY. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, NY. 1994; Long term culture initiating cell assay, Sutherland, H.J. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, NY. 1994.

Tissue Growth Activity

A protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair and replacement, and in the treatment of burns, incisions and ulcers.

A protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced

craniofacial defects, and also is useful in cosmetic plastic surgery.

A protein of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein of the present invention is tendon/ligament formation. A protein of the present invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or

ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide an environment to attract tendon or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors ex vivo for return in vivo to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The protein of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, i.e. for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a protein may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular

diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the invention.

Proteins of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

It is expected that a protein of the present invention may also exhibit activity for generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring to allow normal tissue to regenerate. A protein of the invention may also exhibit angiogenic activity.

A protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

A protein of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for tissue generation activity include, without

limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491 (skin, endothelium).

Assays for wound healing activity include, without limitation, those described in: Winter, Epidermal Wound Healing, pps. 71-112 (Maibach, HI and Rovee, DT, eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, J. Invest. Dermatol 71:382-84 (1978).

Activin/Inhibin Activity

A protein of the present invention may also exhibit activin- or inhibin-related activities. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins and are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a protein of the present invention, alone or in heterodimers with a member of the inhibin α family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the protein of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin-β group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, United States Patent 4,798,885. A protein of the invention may also be useful for

advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for activin/inhibin activity include, without limitation, those described in: Vale et al., Endocrinology 91:562-572, 1972; Ling et al., Nature 321:779-782, 1986; Vale et al., Nature 321:776-779, 1986; Mason et al., Nature 318:659-663, 1985; Forage et al., Proc. Natl. Acad. Sci. USA 83:3091-3095, 1986.

Chemotactic/Chemokinetic Activity

A protein of the present invention may have chemotactic or chemokinetic activity (e.g., act as a chemokine) for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability

to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W.Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub et al. J. Clin. Invest. 95:1370-1376, 1995; Lind et al. APMIS 103:140-146, 1995; Muller et al Eur. J. Immunol. 25: 1744-1748; Gruber et al. J. of Immunol. 152:5860-5867, 1994; Johnston et al. J. of Immunol. 153: 1762-1768, 1994.

Hemostatic and Thrombolytic Activity

A protein of the invention may also exhibit hemostatic or thrombolytic activity. As a result, such a protein is expected to be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds

resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as, for example, infarction of cardiac and central nervous system vessels (e.g., stroke).

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet et al., J. Clin. Pharmacol. 26:131-140, 1986; Burdick et al., Thrombosis Res. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

Receptor/Ligand Activity

A protein of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of

receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for receptor-ligand activity include without limitation those described in:Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W.Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1-7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987; Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenborg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

Anti-Inflammatory Activity

Proteins of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell-cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production of other factors which more directly inhibit or promote an inflammatory response. Proteins exhibiting such activities can be used to treat inflammatory conditions including chronic or acute conditions), including without limitation inflammation associated with infection (such

as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of ytokines such as TNF or IL-1. Proteins of the invention may also be useful to treat anaphylaxis and hypersensitivity to an antigenic substance or material.

Tumor Inhibition Activity

In addition to the activities described above for immunological treatment or prevention of tumors, a protein of the invention may exhibit other anti-tumor activities. A protein may inhibit tumor growth directly or indirectly (such as, for example, via ADCC). A protein may exhibit its tumor inhibitory activity by acting on tumor tissue or tumor precursor tissue, by inhibiting formation of tissues necessary to support tumor growth (such as, for example, by inhibiting angiogenesis), by causing production of other factors, agents or cell types which inhibit tumor growth, or by suppressing, eliminating or inhibiting factors, agents or cell types which promote tumor growth

Other Activities

A protein of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily

characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or caricadic cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional factors or component(s); effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein.

CLAIMS

1. A protein containing the amino acid sequence represented by Sequence No. 1.

2. The protein described in Claim 1 containing the amino acid sequence represented by Sequence No. 2.

3. A cDNA containing the base sequence represented by Sequence No. 3.

4. The cDNA described in Claim 3 containing the base sequence represented by Sequence No. 4.

5. The cDNA described in Claim 3 or Claim 4 which comprises the base sequence represented by Sequence No. 5.

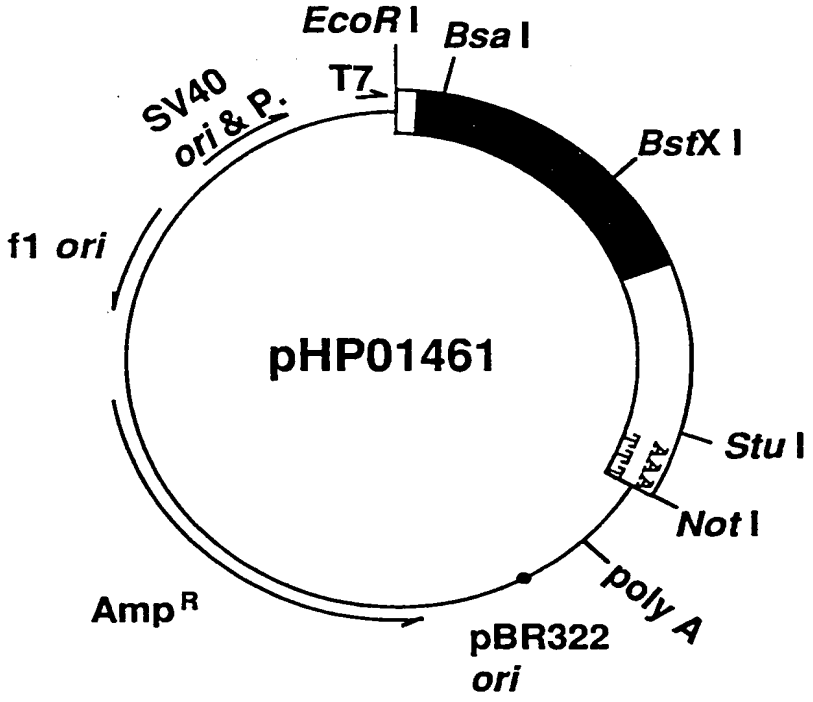
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Figure 1

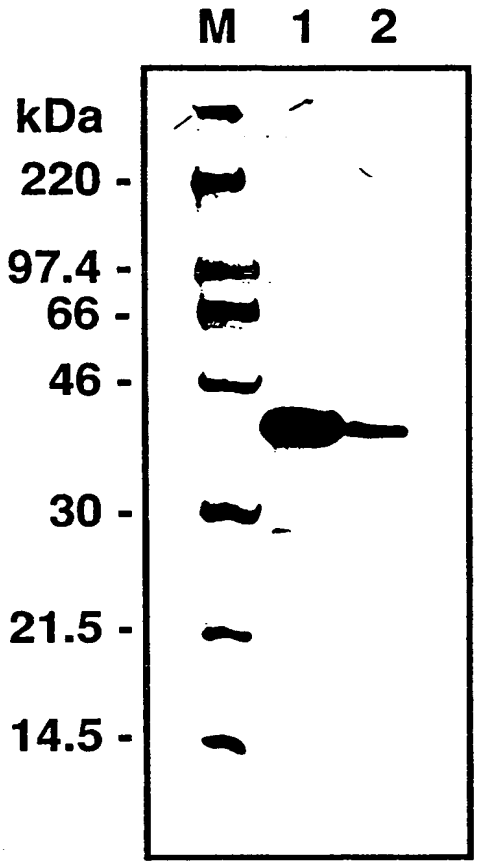
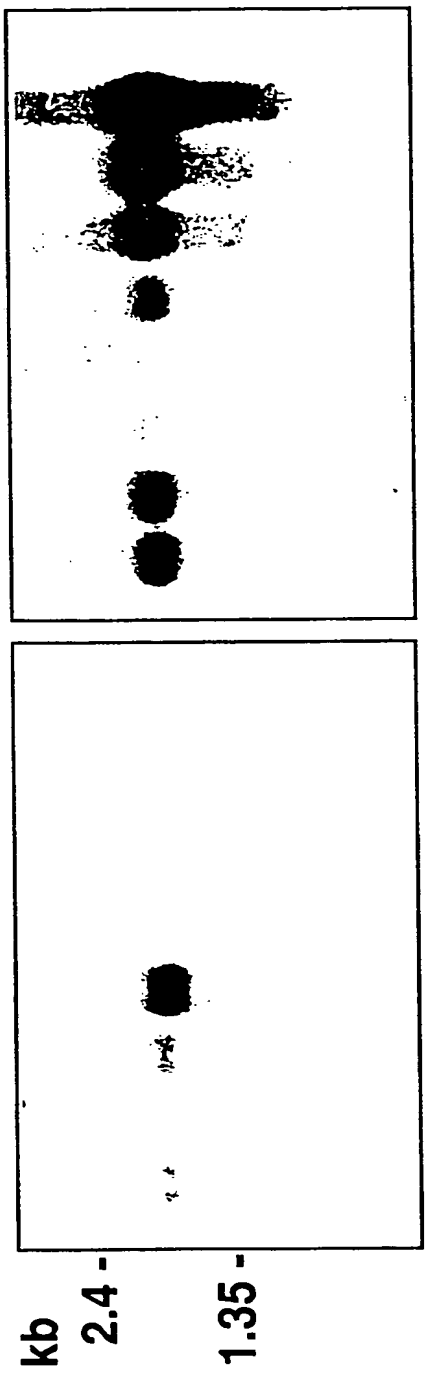


Figure 2



1, Heart; 2, Brain; 3, Placenta; 4, Lung; 5, Liver;

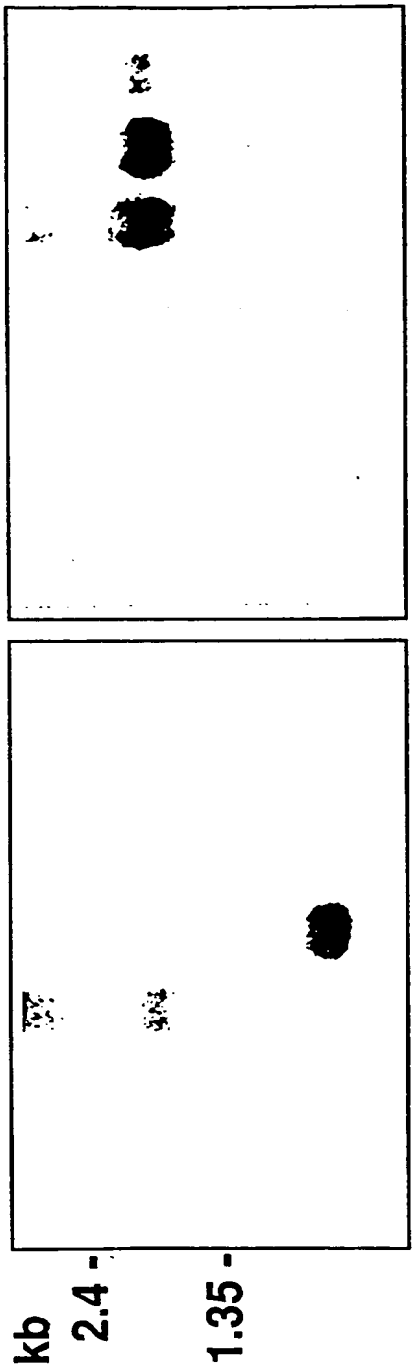
6, Skeletal muscle; 7, Kidney; 8, Pancreas;

9, Spleen; 10, Thymus; 11, Prostate Gland;

12, Testis; 13, Ovary; 14, Small Intestine;

15, Large Intestine; 16, Peripheral Blood Leukocyte

Figure 3



1, Heart; 2, Brain; 3, Placenta; 4, Lung; 5, Liver;
6, Skeletal muscle; 7, Kidney; 8, Pancreas;
9, Spleen; 10, Thymus; 11, Prostate Gland;
12, Testis; 13, Ovary; 14, Small Intestine;
15, Large Intestine; 16, Peripheral Blood Leukocyte

Figure 4

Sequence Listing

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Ser Trp Gly Pro Glu Glu Arg Lys Thr His Met Pro Phe Gln Lys Gly

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/JP 98/03670

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TURECI O ET AL: "Molecular definition of a novel human galectin which is immunogenic in patients with Hodgkin's disease." JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 MAR 7) 272 (10) 6416-22. JOURNAL CODE: HIV. ISSN: 0021-9258., XP002065487 United States cited in the application see the whole document	1-5

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 January 1999	22/01/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kania, T

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WADA J ET AL: "Identification and characterization of galectin - 9, a novel beta-galactoside-binding mammalian lectin." JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 FEB 28) 272 (9) 6078-86. JOURNAL CODE: HIV. ISSN: 0021-9258., XP002089578 United States cited in the application see page 6082, column 2, line 11FF.; figure 1B	1-5
A	WADA J ET AL: "Developmental regulation, expression, and apoptotic potential of galectin - 9, a beta-galactoside binding lectin." JOURNAL OF CLINICAL INVESTIGATION, (1997 MAY 15) 99 (10) 2452-61. JOURNAL CODE: HS7. ISSN: 0021-9738., XP002089579 United States see the whole document	1-5
A	WO 97 03190 A (KATO SEISHI ;SEKINE SHINGO (JP); KAMATA KOUJU (JP); SAGAMI CHEM RE) 30 January 1997 cited in the application see the whole document	1-5
P,X	KATO S.: "AC 014532" EMBL DATABASE,1 January 1998, XP002089580 Heidelberg see the whole document	1,2
P,X	KATO S.: "AC AB006782" EMBL DATABASE,10 September 1997, XP002089581 Heidelberg see the whole document	3-5

INTERNATIONAL SEARCH REPORT

nformation on patent family members

Inter ›nal Application No
PCT/JP 98/03670

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9703190 A	30-01-1997	EP 0841393 A	13-05-1998